rectly in tissue cultures of black and red melanophores.

Reply by Medawar.

The question of whether "infected" red melanophores contain both red and black pigmentary systems, or black alone, is certainly of first-rate importance. Although we are not very optimistic about the use of tissue culture, we hope that really detailed histological analysis will help to solve the problem.

Atypical Pigment Cell Differentiation in Embryonic Teleostean Grafts and Isolates.

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The occasional development of red blood corpuscles and of chromatophores is a condition which sometimes obtains in otherwise nondifferentiating isolates and grafts from young teleostean embryos (Oppenheimer, 1949). Examples of such atypical differentiation have occurred in three separate series of experiments (Oppenheimer, 1936a, 1938) to be discussed below.

Fundulus germ-ring grafts. In this series of experiments, portions of the germ-ring located 90° or 180° from the midline of the embryonic shield of *Fundulus heteroclitus* gastrulae (cf. fig. 1B, Oppenheimer, 1938) were grafted to the embryonic shield or to the extra-embryonic membrane of gastrulae of the same species. In 37 cases either the whole grafts or portions of them continued development but failed to undergo typical histogenesis and differentiated no axial structures. In 16 of these, however, red blood corpuscles differentiated, and in 10 of these 16 grafts melanophores differentiated. In some cases, only a single melanophore was differentiated within the grafts, in others a larger number was present. Only those grafts are included among the 10 positive cases which contained melanophores located more or less centrally in the mass of cells; grafts to which melanophores were applied only at the surface are excluded from consideration in the possibility that in these cases host melanophores might have migrated to cover the grafts.

Only heteroplastic transplantation or the grafting of materials from vitally stained embryos can furnish direct proof that the melanophores are formed by graft rather than host cells in such experiments as these. Fortunately, however, supplementary data are available from other types of experiment which demonstrate that chromatophores are also similarly differentiated in some cases by isolates rather than transplants from young teleostean embryos.

Epiplatys germ-ring isolates. The embryos of *Epiplatys fasciolatus* can be divided at late gastrula stages in such a way (cf. fig. 1A, Oppenheimer, 1938) that the portion of the germ-ring most remote from the embryonic shield can be isolated with part of the yolk from the portion of the egg containing the shield. Such isolates develop for several days in Ringer's solution. Though in some cases they exhibit certain phenomena of growth which simulate the form of the tail, they undergo no histogenesis of axial structures. Of 12 such aggregates studied, one developed red blood corpuscles, one formed a single melanophore and a third differentiated a number of xanthophores. One explant of prospective tailbud region, isolated immediately after the closure of the blastopore, formed both melanophores and xanthophores although otherwise undifferentiated.

Fundulus isolated blastoderms. Blastoderms of *Fundulus heteroclitus* separated from the yolk during cleavage stages and cultivated in double-strength Holtfreter's solution under some conditions form hyperblastulae, masses of nondifferentiated cells provided with a large vesicle at one pole (Oppenheimer, 1936a). Of approximately 75 hyperblastulae studied, only one developed a single melanophore of typical size and configuration.

Discussion and conclusions. The production of chromatophores by the grafts and isolates described above suggests that in the teleosts under certain experimental conditions pigment cells can be differentiated by cells which normally do not contribute to the teleostean counterpart of the neural crest. The 90° germ-ring of *Fundulus* normally contributes primarily mesoderm to the embryo (Oppenheimer, 1936b); and the 180° germ-ring is unrelated to nervous system formation except insofar as it contributes to the tailbud blastema from which neural derivatives may later arise. While it is theoretically possible, it is highly improbable that the chromatophores in these cases have been differentiated only by the particular cells destined to form them later after their passage through the tailbud blastema. The differentiation of pigment cells under these conditions is therefore presumably the result of what the experimental embryologists call *bedeutungsfremde Selbstdifferenzierung.*

This interpretation, however, is not incompatible with the possibility that in teleosts the chromatophores normally arise from cells corresponding to those of the neural crest of other vertebrates, as suggested by the results of the transplantation experiments of Lopashov (1944) on three species of teleosts, and in line with the neural crest

origin of pigment cells in amphibians (DuShane, 1935), birds (Dorris, 1938) and mammals (Rawles, 1947). It merely signifies that under certain abnormal conditions, other than the usual cells can take over the function of pigment cell formation.

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Regeneration of Neural Retina and Lens from Pigment Cells in the Eyes of Adult Salamanders.*

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The neural retina of adult salamander eyes degenerates when deprived of its blood supply either by severing the vessels which vascularize it (Stone and Chace, 1941) or by transplanting the entire eye (Stone and Zaur, 1940; Stone and Cole, 1943; Stone and Ellison, 1945). The only retinal cells which survive are those of the pigmented epithelial layer from which a new neural retina regenerates (Stone, 1949). This has been studied in detail in many grafted adult urodele eyes. Degeneration spreads throughout the neural retina during the first three weeks. It often proceeds at different rates in various parts of the same eye, allowing early stages of regeneration to begin in some areas while adjacent regions are still degenerating. The true origin of the new neural retina is therefore more easily followed in cases where degeneration is simultaneous in all parts or where the entire neural retina is detached from the underlying retinal pigment layer by a gentle stream of Ringer's solution and then removed intact through a broad dorsal slit in the eye.

Degeneration of the neural retina in grafted eyes usually spreads rapidly until the retinal pigment cells are finally denuded and the cellular debris is absorbed above them. The pigment cells then become dense black oval or flattened bodies resembling those found soon after surgical removal of the entire neural retina. Now they take on temporarily another function, entering the critical stages which can be followed step by step as they determine the origin of the new neural retina.

These pigment cells increase markedly in size. Their nuclei become distinct as they undergo mitosis. One daughter cell migrates inward, loses its pigment and with similar ones forms a sharply defined layer, which by further cell division gives rise to a new neural retina. The other daughter cell retains its pigment and later takes on the status of a retinal pigment cell when the neural retina above it has fully regenerated. The functional capacity of this cell is finally expressed by the migration of its pigment granules in a light-adapted eye as soon as the rods and cones above it are differentiated.

When a small portion of the neural retina is excised, or is detached as a permanent elevated fold, regeneration is also called forth from the underlying retinal pigment cells. On the other hand the initial reaction of the pigment cell to a small retinal injury is one of rapid mitosis and mass migration into the wound, somewhat similar to that found in the retinal wounds of other vertebrates. However, in the urodele retina these cells later lose their pigment and differentiate into neural retinal tissue. Retinal pigment epithelium transplanted into the eye chambers also gives rise to neural retina.

Since the early pigment cell changes involve replacement of a lost tissue the term "dedifferentiation" might be considered as it applies to them. This term is often loosely and obscurely applied to cells which are assumed to be taking part in regeneration, although up to that moment they are recognized as highly differentiated elements with special morphological and functional characteristics. Under the proper stimulus they are supposed to lose their special features and take on a role of supplying new cells that will later develop into similar or different elements. If the term is defined in this sense it can be said that the retinal pigment cell in the salamander eye is capable of dedifferentiation.

Another pigment cell in the eye of *Triturus* salamanders may be considered in the same category, for when the lens is removed the

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